# Lab Recap: Restriction Digest Gels (RDG)

*The Moment of Truth Part II*



**Purpose:** To run RD and MP samples through a gel in order to obtain information about your insert.

**Vocabulary/Terms:**

***Gel Electrophoresis*** - Running a current through a gel that is loaded with DNA samples in order to separate DNA fragments based on size.

***Standard*** (also known as Marker or Ladder) - A collection of DNA fragments of known sizes. You will compare this to your own duckweed DNA sample to approximate the size of your duckweed insert. $tandard is very expen$ive so handle it with care. Color = dark blue.

***Loading Dye*** - What you put in your PCR sample before you put it into the gel. It helps you see the sample in the gel (gives it blue color) and ensures that your DNA will not flow out of the well. This looks a lot like standard, so don't confuse the two. Color = dark blue.

***Agarose*** - The sugar molecules that make up the gels we are using. Smaller DNA fragments can pass through them in a gel relatively quickly. Large DNA fragments have trouble.

***Stain*** - We we put in your gels so that you can see the bands under UV light. The stain adds radioactive tags to DNA and the tags glow under UV light. Since the stain adds radioactive things to DNA, it is considered a carcinogen. If the stain can add radioactive things to duckweed DNA, then it can add radioactive things to you as well--which is why you should never touch a gel without gloves. The stain we used it called Ethidium Bromide (EtBr) and you will most likely never see it unless you come in early to make gels. Color = red

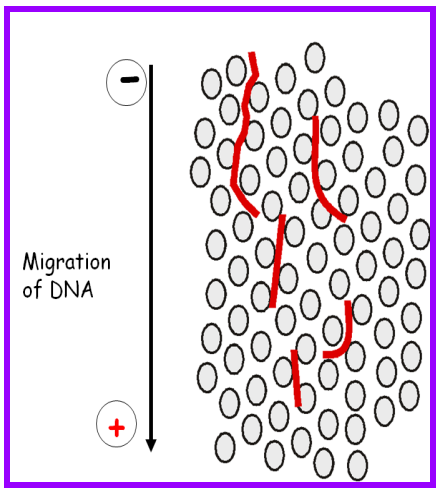
***Buffer*** - Something that regulates pH. Your gels are sitting in buffer.

***Cut/Digest*** - The DNA that has been cleaved (cut) by the PvuII-HF restriction enzyme. As a result, the tube with your cut/digested DNA contains your insert plus a few bases separated from the vector backbone. This is the sample you made in the RD lab. It will be in the "D" tube, for "digest"

***Uncut*** - DNA from your miniprep tube...AKA straight-up plasmid DNA. It has not been cut by anything, hence the name uncut.

**Concept: Gel Electrophoresis**

- We create an electric current by putting a negative (black) electrode on the top and a positive (red) electrode at the bottom
- DNA is negatively charged
- DNA is repelled by the negative electrode and attracted to the positive electrode, therefore it migrates down the gel
- Shorter fragments move farther because it is easier for them to pass through the agarose. Larger fragments do not move as much because it is harder to pass through the agarose.



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**What you will be doing and why you will be doing it**

Before this lab:

1. You purified (separated from unwanted cellular parts) your plasmid DNA in the miniprep lab
2. You cut your plasmid DNA using restrictions enzymes and placed the resultant into the "D" tube in the restriction digest lab

During this lab:

1. You will be prepping an uncut DNA tube by taking DNA from your miniprep tube and adding loading dye + water
2. You will then be loading a gel: Adding standard to a well, adding your uncut DNA to another well, and adding your cut (digested) DNA to another well
3. You will run a current through the gel and then as a class, we will look at all of the gels under UV light. We will take pictures of the gels and you will further analyze them later.

Why you are doing it:

1. To give another way to predict the (1) size and (2) quality of our DNA sample
2. If you messed up PCR or PCRG, you can use this gel to determine size, quality, and "sequencability" of your insert. It's like a little life-saver
    a. We'll get into all of the great stuff you can learn about your clone and insert in the next recap. For now, we're just concerned with you making your uncut sample and loading the gel properly. We're saving all the fun stuff for the analysis--the mockup recap.

**RDG is the moment of truth (Part II)**

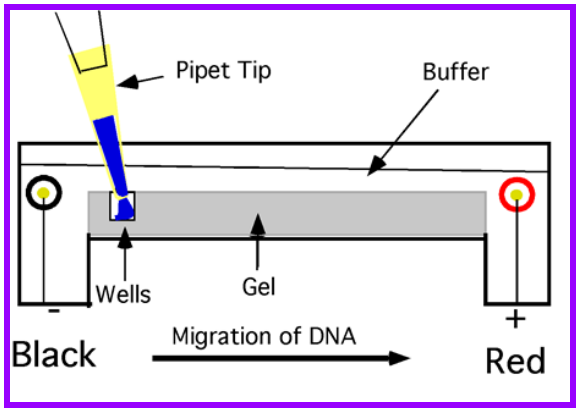
- Part I = PCRG
- This is your *last* chance before sequencing to find out some information about the duckweed DNA that was originally locked inside bacteria. Exciting, right?
- If you messed up the miniprep lab, it will be evident
- If you messed up the restriction digest lab, it will be evident

**Q&A:**

Q: Any tips for loading a gel?

A: Make sure to:

- Not confuse standard with loading dye
- Pipet out your sample when you are near the top of the well so you do not puncture the gel
- Put a finger on your pipet or put your elbows on the table to help stabilize yourself
- Go down to the first stop when you are pipetting liquid into the well. This is the only time you go down to the first stop when you are removing something from your pipet (you usually go down to the second stop). Then, make sure you let go of the pipet when the tip is outside the gel so that you do not suck up any PCR samples in the well



Q: Why do we need cut and uncut DNA?
A:

- To see if the enzyme actually cuts and to compare the sizes
- If the cut and the uncut are the same, then something went wrong
    - The digest (the "cutting" of your plasmid) did not work and your RD gels cannot tell you the size of your insert
    - Uncut lane = *negative control*
- This will all make more sense when you do your RD mockup

Q: What happens if my samples float out of the well when I pipet them in?
A: LOL.

- That means that for whatever reason, there is still ethanol in your prep--probably because you forgot to do the second spin in the wash buffer step of the miniprep lab.
    - Remember, wash buffer has ethanol--so forgetting to do that second spin means that you have leftover wash buffer → you have leftover ethanol.
- The ethanol is less dense than the buffer that the gels are submerged in. So, if you have ethanol in your prep, your samples will float out of the wells, regardless of if you added loading dye.
- To make matters worse, ethanol interferes with sequencing reactions so your prep won't be sequenced if it has ethanol in it.
- So, if you have ethanol in your preps,
    1.) Your DNA will float out of your wells
    2.) Your DNA wont be sequenced
    3.) You won't get published.
- In other words, YOU'RE SCREWED!!!!!!!!!!

**Video** (use DSAP login to access)
How to load a gel (skip to 2:24): https://wssp.rutgers.edu/WSSP-files/Videos/Gel_Loading.mov

*****UNDERSTAND that this is basically the same thing as the PCRG lab, except that**

1. You're making an uncut DNA tube instead of adding loading dye to your PCR tube
2. You're loading two samples per person instead of one,
3. Mistakes from the miniprep lab can resurface during this lab and prevent you from loading your gel properly (i.e. ethanol in your prep)